PATENT ABSTRACTS OF JAPAN

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(54) FUNCTIONAL FOOD COMPOSITION

(57)Abstract:
PURPOSE: To provide a food raw material effective in suppressing the increase of blood pressure caused by salt and having a function to lower the cholesterol level in blood when taken together with salt.
CONSTITUTION: A peptide having angiotensin converting enzyme inhibiting action and obtained by the hydrolysis of corn protein is added to a food to be taken together with salt. The purpose of the invention can be achieved by this process.

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* NOTICES *

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CLAIMS

[Claim(s)]
[Claim 1] The functional food constituent characterized by containing the peptide which has the angiotensin conversion enzyme inhibition activity acquired by hydrolyzing maize protein.
[Claim 2] The functional food constituent which comes to contain sodium salt with the peptide which has the angiotensin conversion enzyme inhibition activity acquired by a proteolytic enzyme decomposing maize protein.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the constituent for functional food effective in prevention of hypertension or hyperlipidemia, or an improvement of these many symptoms.
[0002]
[Description of the Prior Art] The therapy or prevention -- complication with the hyperlipidemia which is the disease which hypertension is one of the diseases which occupies the high order of the death rate in our country, and can be said to be that the present-day lifestyle has brought further is made into a problem today -- has been an urgent and important technical problem.
[0003] There are secondary hypertension which can specify a cause, and essential hypertension which cannot specify a cause in hypertension, and it is said that the 75% is the latter. Various-causes children, such as overcatecholamine secretion by superfluous intake of salt, the hereditary factor, renin angiotensin aldosterone accommodative insufficiency, and the sympathicotonia, are pointed out to the onset mechanism of essential hypertension, and it is supposed that these cause the onset in relation to independent or mutual. Administration of the drug which adjusts a nerve factor although medication is generally carried out to the therapy of this essential hypertension is ****** expected the therapy by accommodation of the renin angiotensin aldosterone series which is a body fluid sex factor since the side effect is large.
[0004] On the other hand, although a low salt diet thing therapy is mentioned as one of the non-pharmacotherapy, making the palatability which participates in the appetite which is one of the human appetition reduced is accompanied by very strong pain. Then, even if it takes in salt etc., an appearance of the food which can control the elevation of blood pressure under the effect is desired strongly.
[0005]
[Problem(s) to be Solved by the Invention] To the man of hypertension or a hypertension inclination, this invention person etc. took in to a high sodium inclusion and coincidence, such as salt, and inquired wholeheartedly that the ingestion constituent which mitigates the vasopressor activity should be offered. Consequently, the thing for which the peptide which has the angiotensin-converting-enzyme (it is hereafter written as ACE) inhibition activity acquired by hydrolyzing the protein of the corn origin where it is taken in daily and safety is checked has the operation, Furthermore, possibility of the operation concerned being imagined to be what is depended on ACE inhibition while doing research detailed about the operation mechanism, and discovering the moderate blood-pressure drop operation based on accommodation of a body fluid sex factor is very high, And this matter came to complete a header and this invention for having the cholesterol lowering operation in blood.
[0006]
[Elements of the Invention] That is, it is the functional food constituent characterized by this invention containing the peptide which has the angiotensin conversion enzyme inhibition activity acquired by hydrolyzing maize protein, and is the functional food constituent characterized by containing the sodium salt like salt with this peptide further.

[0007] As a peptide which has the ACE inhibition activity acquired by hydrolyzing the maize protein said to this invention, it is Agric.Biol.Chem., 53 (4), 1077-1081 (1989), Agric.Biol.Chem. and 55 (5), and 1313-1318 (1991), A thing as indicated, and it is well-known, for example, shown below can be mentioned. Leu-Arg-Pro, Leu-Ser-Pro, Leu-Gln-Pro, Leu-Pro-Pro, Leu-Ala-Tyr, Ile-Arg-Pro, Val-His-Leu-Pro-Pro, Leu-Thr-Pro, Val-Ser-Pro, Leu-Asn-Pro, Leu-Leu-Pro, Ile-Arg-Ala, Val-Ala-Tyr, Leu-Ala-Ala, Val-Ala-Ala, Leu-Gln-Gln, Ile-Arg-Ala-Gln-Gln, Phe-Tyr.
[0008] As maize protein used for manufacture of this peptide, it is the manufacture process of the corn starch by the wet milling method, for example, and the so-called "zein" etc. extracted and obtained with alcohol or alkali can mention the suspension (cone gluten meal suspension) or gluten meal, and corn particle of the maize protein which is immersed in a sulfurous-acid water solution and obtained in a corn grain.
[0009] Although the peptide concerning this invention is obtained by hydrolysis according said maize protein to an enzyme, acids, or alkali, its approach by the enzyme which can choose mild conditions especially as a reaction is advantageous. It will be as follows if an example of the decomposition approach by the enzyme which uses a gluten meal as a raw material is given as a process of this peptide. That is, water is made to carry out distributed suspension and a gluten meal adds the enzyme chosen from the enzyme agent containing the proteinase which hydrolyzes association inside protein peptide chains, such as thermolysin, SAMOAZE, a papain, a trypsin, alpha-chymotrypsin, and subtilisin, to this. The concentration of a substrate or an enzyme, pH of reaction mixture or temperature, and other conditions do not require especially special actuation that what is necessary is just to choose the conditions optimal for each enzyme agent. For example, Agric.Biol.Chem., 55 (5), and 1407-1408 (1991), The indicated approach is employable as it is.
[0010] The peptide generated by decomposition is refined by the ultrafiltration, adsorption material processing, and the other proper well-known approaches remaining as it is or if needed, and is further dried by the proper desiccation approach of spray drying, freeze drying, and others as occasion demands. If the peptide which has the ACE inhibition activity which hydrolyzed and obtained the maize protein of this invention like the above can be used for the extensive food which includes animal feed, can demonstrate the functionality and shows the example, it can mention various drinks, such as juice, panconfectionary, frozen desert, dressings, a water zootechnics surimi product, soup, a seasoning, etc. This peptide does not need exceptional process modification or caution at all that what is necessary is just to add at the process of the arbitration in manufacture of these food.
[0011] Furthermore, this peptide has the remarkable effectiveness which mitigates an operation of the sodium salt to hypertension, if it takes in with the food containing sodium salt. Soup powder, such as vegetable juice, such as food processed using seasonings, such as salt, bean paste, soy sauce, and sodium glutamate, and these as an example of sodium salt content food or luxury goods, for example, virgin bloody Mary etc., pickles, a dried food, picked fish guts, food boiled down in soy, butter, mayonnaise, a sausage, confectionary, nuts, cup soup, and instant noodles, etc. is mentioned here.
[0012] Although physiological functions, such as said blood-pressure drop operation, are demonstrated as long as the above-mentioned peptide concerning this invention takes in this, intake is usually 1g of sodium salt in food. 4g [5mg -] of hits The range is suitable and a proper amount is chosen from this range according to the class and the intake object of food.
[0013]
[Test Example(s)]
Example 1 of manufacture Zein (Wako Pure Chem make) 750g is suspended in 15l. of 4-degree C deionized water among the jar fermenter (product made from KEMAPPU) of 20 liter capacity, and it is pH by 25% aqueous ammonia (Wako Pure Chem make) 7.5 It prepares. The reaction was performed at 60 degrees C for 18 hours, adding and stirring thermolysin (Made in formation [Yamato]) 7g. After carrying out the autoclave (105 degrees C, 5 minutes) of this and carrying out deactivation of the thermolysin, centrifugal separation (6,000rpm, 15 minutes) is carried out, and it is pore size further. It freeze-dries by a 0.2-micron membrane filter removing a solid, and is peptide powder. 745g It obtained.
[0014] Example 1 of a trial The rabbit langue acetone powder (sigma company make) of 5g of

measurement of ACE inhibition activity was dissolved in the 50ml 0.1M boric-acid buffer solution (pH 8.3), and at-long-intervals alignment separation was carried out by 40,000 x g for 40 minutes. The supernatant liquor was diluted with the above-mentioned buffer solution 10 times, and angiotensin-converting-enzyme liquid was obtained. 0.03ml of test liquids is taken in a test tube, and it is a substrate to this. The above-mentioned enzyme liquid after adding the HIPURIRU-L-histidyl-L-leucine (sigma company make, last concentration 5mM, sodium chloride 300mM is included) of 250 microliter and pre incubating for 10 minutes at 37 degrees C 0.1ml added and it was made to react for 30 minutes at 37 degrees C. the passage of time of after, and 1 N 0.25ml of hydrochloric acids is added and a reaction is stopped -- 1.5ml ethyl acetate was added and it was made to stir violently for 15 seconds Then, centrifugal was carried out for 15 minutes by 3,500rpm, and 1ml of ethyl acetate layers was extracted. Reduced pressure clearance of the solvent was carried out from the ethyl acetate layer. Absorption (absorbance of 228 nm) of the HIPURIN acid which added 1ml of distilled water and was extracted was measured after solvent clearance, and inhibition activity was searched for from the following formulas. Sample concentration which shows 50% of rate of inhibition was set to IC50.
Rate of inhibition =/(A-B) A x 100 %, however A : Absorbance B of 228nm in case an inhibitor is not included : IC50 of the ACE inhibition activity of the hydrolyzate of the maize protein which was prepared by the example 1 of the absorbance aforementioned manufacture of 228 nm in inhibitor addition was 21 micrograms/ml.
[0015] The spontaneously hypertensive rat was made to carry out free intake of the feed containing the hydrolyzate and salt of the maize protein prepared in the example 1 of example of trial 2 manufacture, and elevation-of-blood-pressure depressant action was seen. The healthy thing was used for the trial after carrying out preliminary breeding of the spontaneously hypertensive rat (Charles River Japan, INC.) of 3 weeks old for one week at the breeding room of the temperature of 23 ** 3 degrees C, and 50**10% of humidity. Trial feed is 10% of 28% [of hydrolyzates of the maize protein obtained in the example 1 of manufacture] (24.6% as amount of crude protein), corn-starch 41.5%, and alpha starch, cellulose powder 8%, 6% of vegetable oil, 3.5% of minerals, and 5% of granulated sugar. And the "peptide mixed feed" which mixed 1% of vitamins was prepared, and this and "MF powder feed" by Oriental Yeast Co., Ltd. were mixed by different ratio. That is, only "MF powder feed" is (a control plot 1), the thing (experimental plot 1) by which "MF powder feed" and "peptide mixed feed" were blended with 2:1, and the thing (experimental plot 2) which blended this forward one with 1:1. One experimental plot made the duration of test four weeks using seven rats each. each -- access -- feed used the amount of salt, adjustment and drinking water used tap water to 3.2%, and all were made to take in freely Measurement was performed once at one week per the amount of feed intake, a gain-of-body-weight pile, blood pressure, and heart rate. Measurement of blood pressure and a heart rate calculated the average of the value measured 6 times per animal using bloodless blood pressure and a heart rate measuring device (TK[by Unicom Corp.]-350 mold). A test result shows a blood-pressure-measurement result to drawing 1 , feed intake, and a gain-of-body-weight pile list, and shows the measurement result of a heart rate in a table 1, respectively.
[0016] In the experimental plot 1 and experimental plot 2 which paid the feed which the spontaneously hypertensive rat of a control plot indicated that remarkable elevation of blood pressure was clear from drawing 1 by superfluous intake of salt, on the other hand blended the corn peptide of this invention, the lifting depressor effect of significant blood pressure was accepted.
[0017]
[A table 1] On the other hand, the difference with any capable division was not accepted that a heart rate is looked at by the table 1. Moreover, in a table 1, there is no difference also with the amount of feed intake and a gain-of-body-weight pile significant in each section, and it was checked that a nutrition side and a safety aspect are also satisfactory.
[0018] While making the spontaneously hypertensive rat of advanced age carry out free intake of the feed containing the hydrolyzate and salt of the maize protein prepared in the example 1 of example of trial 3 manufacture more and checking elevation-of-blood-pressure depressant action, the effect on a blood cholesterol level etc. was investigated. The healthy thing was used for the trial after carrying out

preliminary breeding of the spontaneously hypertensive rat (Charles River Japan, INC.) of 6 weeks old for one week like the example 2 of a trial. Feed should blend the thing "MF powder feed" (control plot 2) by Oriental Yeast Co., Ltd., and the peptide obtained by this in the example 1 of manufacture to 10% (experimental plot 3), and both final concentration Salt was added so that it might become 3.2%. Drinking water was made to carry out free intake of each tap water. Measurement of the amount of feed intake, a gain-of-body-weight pile, blood pressure, and a heart rate was performed like the example 2 of a trial. Moreover, after test termination, decapitation of the rat was carried out, after extracting blood, promptly, the quantum of angiotensin II of blood was performed by the radioimmunoassay method, and the quantum of total cholesterol, neutral fat, and high density lipoprotein cholesterol was performed with enzymatic process. A test result shows the measurement result of a heart rate to drawing 2 , feed intake, and a gain-of-body-weight pile list, and shows the measured value of angiotensin II in a table 2 and blood, the total cholesterol in blood, neutral fat, and high density lipoprotein for a blood-pressure-measurement result in a table 3, respectively.

[0019] By the trial group which paid the feed which the spontaneously hypertensive rat of a control plot indicated that remarkable elevation of blood pressure was clear from drawing 2 by superfluous intake of salt, on the other hand blended the corn peptide of this invention, the lifting depressor effect of significant blood pressure was accepted.

[0020]

[A table 2]

[0021]

[A table 3] The difference significant to a control plot and the test section did not have private seals in the amount of feed intake, and a gain-of-body-weight pile list like [a heart rate] the example 2 of a trial.

(A table 2)

Although it was increasing remarkably in the control plot as the amount of plasma angiotensin II was shown in a table 3, it fell intentionally by combination of the peptide of this invention, and the level was 1/3 or less [of a control plot]. Furthermore, it turned out that these are decreased intentionally, without having accepted the lowering effectiveness in the division which added the corn peptide of this invention, and accompanying both the total cholesterol and amounts of neutral fat in blood by lowering of useful HDL cholesterol for a living body.

[0022]

[Example] An example is shown below.

Example 1 The corn peptide of 0.5% per weight of example 1 of manufacture was added to the vegetable juice (0.8% of salt contents) of vegetable juice marketing, it dissolved in it, and the vegetable juice of this invention was made.

Example 2 The corn peptide of 5% per weight of example 1 of manufacture was scoured for the Shinshu bean paste (about 11% of salt contents) prepared in the bean paste usual manufacturing method, and the bean paste of this invention was made.

Example 3 The peptide of 5% per weight of example 1 of manufacture was added and produced to the powdered soup (about 55% of salt contents) of instant noodles prepared in the instant-noodles soup usual manufacturing method.

[0023]

[Effect of the Invention] Especially the functional food constituent that was made to contain the peptide which has the angiotensin conversion enzyme inhibition activity acquired by hydrolyzing maize protein by this invention, and was obtained has the function to suppress the vasopressor activity which sodium salt has, and to reduce the cholesterol in blood, and the amount of neutral fat intentionally, by using with sodium salt, such as salt, in common. Therefore, it has a hope great to prevention for the therapy list of the hyperlipidemia accompanying hypertension or this in the functional food constituent of this invention.

[0024]

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It reaches.
[Drawing 2] The average and standard deviation for every group show the blood-pressure-measurement result of ** and an animal experiment [in / respectively / the example 2 of a trial, and the example 3 of a trial], an axis of abscissa is the week-old of a rat, and all of an axis of ordinate are the systolic blood pressure (mmHg) of a rat. each line in drawing -- the continuous line in drawing 1 -- in the experimental plot 1 and the dotted line, the continuous line in drawing 2 shows a control plot 2, and the dotted line shows [the control plot 1 and the dashed line] the result of an experimental plot 3 for the experimental plot 2, respectively.
[A table 1] The measurement result after four weeks of administration in the example 2 of a trial (8-weeks old o'clock)

experimental plot	The amount of average feed intake (g/head/day)	an increase (g/4weeks)	--	The body Pile (beats/min)	Alignment **	Number
Control plot	15.7 **	0.6	123.3 **	4.7	457.9 **	15.5
Experimental plot 1	14.3 **	0.8	125.9 **	3.0	442.4 **	9.2
Experimental plot 2	14.7 **	0.6	124.0 **	2.7	434.7 **	10.9

[A table 2] The measurement result -1 after three weeks of administration in the example 3 of a trial (10-weeks old o'clock)

experimental plot	The amount of average feed intake (g/head/day)	an increase (g/3weeks)	--	The body Pile (beats/min)	Alignment **	Number
Control plot 2	16.2 **	0.6	90.6 **	5.6	452.9 **	15.5
Experimental plot 3	16.0 **	0.8	88.4 **	4.8	454.4 **	9.2

[A table 3] The measurement result -2 after three weeks of administration in the example 3 of a trial (10-weeks old o'clock)

Experimental plot	Angiotensin II in blood (ng/ml)	This total cholesterol (mg/ml)	This neutral fat (mg/dl)	This HDL cholesterol * (mg/dl)		
Control plot 2	91.8 **	18.6	55.3 **	2.6	140 **	16 41.3 ** 1.7
Experimental plot 3	27.5 **	5.6	47.0 **	1.5	120 **	16 40.0 ** 0.9

Note * High density lipoprotein cholesterol

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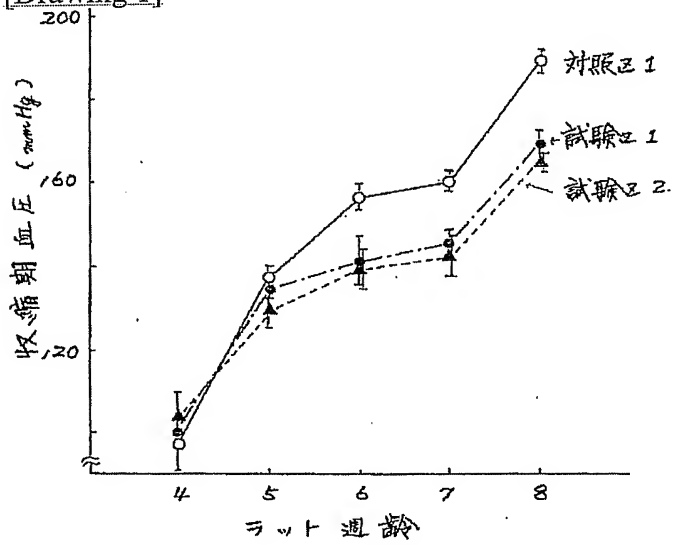
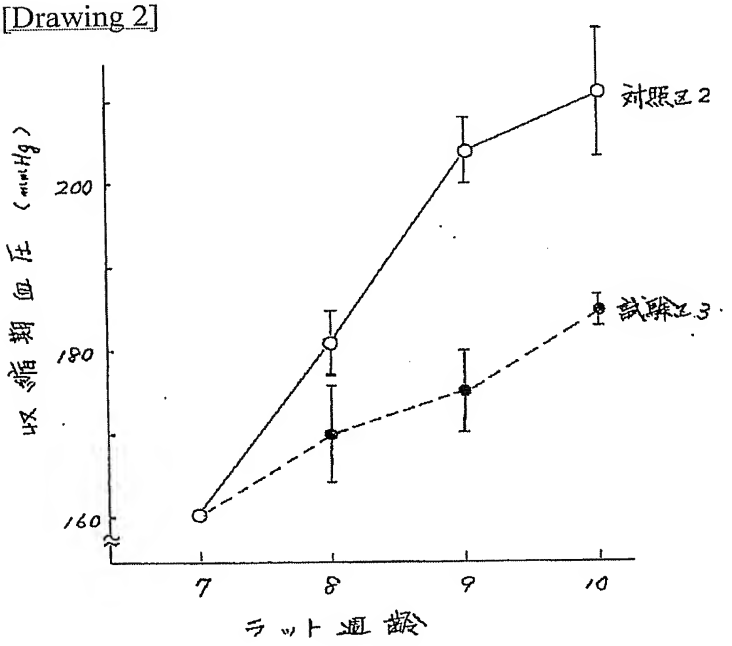
DRAWINGS

[Drawing 1]



[Drawing 2]



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最終頁に続く

(54)【発明の名称】　高脂血症の治療又は予防用経口剤

(57)【特許請求の範囲】
【請求項１】　トウモロコシ蛋白質を加水分解して得られるアンジオテンシン変換酵素阻害活性を有するペプチドを有効成分として含有する高脂血症の治療又は予防用経口剤。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】本発明は高脂血症の治療又は予防用経口剤に関する。
【０００２】
【従来の技術】今日、高血圧症は我国において死亡率の上位を占める疾病の一つであり、さらに現代の生活様式がもたらしているとも言える疾病である高脂血症との合併症が問題とされる等、その治療あるいは予防は緊急かつ重要な課題となっている。

【０００３】高血圧症には、原因の特定できる二次性高血圧症と原因の特定できない本態性高血圧症とがあり、その７５％が後者であるといわれている。本態性高血圧症の発症機序には、食塩の過剰摂取、遺伝性因子、レニン・アンジオテンシン・アルドステロン調節不全、交感神経緊張によるカテコラミン過剰分泌などの諸因子が指摘されており、これらが単独あるいは相互に関連して発症の要因となっているとされる。かかる本態性高血圧症の治療には薬物投与が一般に行われるが、このうち神経因子を調節する薬物の投与は副作用が大きいため、体液性因子であるレニン・アンジオテンシン・アルドステロン系の調節による治療が望まれている。
【０００４】
【発明が解決しようとする課題】本発明者等は、トウモロコシ起源の蛋白質を加水分解して得られるアンジオテ

ンシン変換酵素（以下、ＡＣＥと略記する）阻害活性を有するペプチドに血圧上昇作用を軽減する作用があること、更に、その作用機作について詳細な研究を行う中で、当該作用はＡＣＥ阻害によるものと推察され、体液性因子の調節に基づく穏やかな血圧降下作用を発現する可能性が極めて高いこと、しかも本物質は血液中のコレステロールや中性脂肪の量を低下させることを見出し、本発明を完成するに至った。

【０００５】

【課題を解決するための手段】すなわち、本発明はトウモロコシ蛋白質を加水分解して得られるアンジオテンシン変換酵素阻害活性を有するペプチドを有効成分として含有する高脂血症の治療又は予防用経口剤に関する。

【０００６】本発明に云うトウモロコシ蛋白質を加水分解して得られるＡＣＥ阻害活性を有するペプチドとしては、Ａｇｒｉｃ．Ｂｏｉｌ．Ｃｈｅｍ．，５３（４），１０７７－１０８１，（１９８９）やＡｇｒｉｃ．Ｂｏｉｌ．Ｃｈｅｍ．，５５（５），１３１３－１３１８，（１９９１）に記載されて公知の、例えば以下に示すようなものを挙げることが出来る。Ｌｅｕ－Ａｒｇ－Ｐｒｏ，Ｌｅｕ－Ｓｅｒ－Ｐｒｏ，Ｌｅｕ－Ｇｌｎ－Ｐｒｏ，Ｌｅｕ－Ｐｒｏ－Ｐｒｏ，Ｌｅｕ－Ａｌａ－Ｔｙｒ，Ｉｌｅ－Ａｒｇ－Ｐｏｒ，Ｖａｌ－Ｈｉｓ－Ｌｅｕ－Ｐｒｏ－Ｐｒｏ，Ｌｅｕ－Ｔｈｒ－Ｐｒｏ，Ｖａｌ－Ｓｅｒ－Ｐｒｏ，Ｌｅｕ－Ａｓｎ－Ｐｒｏ，Ｌｅｕ－Ｌｅｕ－Ｐｒｏ，Ｉｌｅ－Ａｒｇ－Ａｌａ，Ｖａｌ－Ａｌａ－Ｔｙｒ，Ｌｅｕ－Ａｌａ－Ａｌａ，Ｖａｌ－Ａｌａ－Ａｌａ，Ｌｅｕ－Ｇｌｎ－Ｇｌｎ，Ｉｌｅ－Ａｒｇ－Ａｌａ－Ｇｌｎ－Ｇｌｎ，Ｐｈｅ－Ｔｙｒ。

【０００７】本ペプチドの製造に使用するトウモロコシ蛋白質としては、例えばウェットミリング法によるコーンスターチの製造過程で、トウモロコシ粒を亜硫酸水溶液に浸漬して得られるトウモロコシ蛋白質の懸濁液（コーングルテンミール懸濁液）、あるいはグルテンミールやトウモロコシ粒子をアルコールまたはアルカリにて抽出して得られる、いわゆる「ツェイン」等を挙げることができる。

【０００８】本発明に係るペプチドは、前記トウモロコシ蛋白質を酵素または酸類、あるいはアルカリ類によって加水分解することにより得られるが、特に反応に穏和な条件を選択できる酵素による方法が有利である。本ペプチドの製法として、グルテンミールを原料とする酵素による分解方法の一例を挙げるならば以下のとおりである。すなわち、グルテンミールは水に分散懸濁させ、これにサーモリシン、サモアーゼ、パパイン、トリプシン、α－キモトリプシン、ズブチリシン等、蛋白ペプチド鎖内部の結合を加水分解するエンドペプチターゼを含む酵素剤から選択される酵素を加える。基質や酵素の濃度、反応液のｐＨや温度、その他の条件は、各酵素剤にとって最適な条件を選択すればよく、とりわけ特殊な操作を要しない。例えば、Ａｇｒｉｃ．Ｂｏｉｌ．Ｃｈｅｍ．，５５（５），１４０７－１４０８，（１９９１）に記載された方法をそのまま採用することができる。

【０００９】分解によって生成したペプチドは、そのまま、あるいは必要に応じ限外濾過、吸着剤処理、その他の適宜の公知の方法で精製し、更に必要により噴霧乾燥、凍結乾燥その他の適宜の乾燥方法で乾燥する。

【００１０】本発明に係るペプチドはそのまま高脂血症の治療又は予防用経口剤として用いることができ、また経口剤用の慣用の製薬補助剤と混合し、高脂血症の治療又は予防用経口剤として製剤化することができる。

【００１１】本発明の経口剤は高脂血症の治療又は予防剤として用いることができるが、この高脂血症は高血圧症を伴う合併症であってもよい。本発明の経口剤の投与量は、ナトリウム塩１ｇ摂取当たり、上記ペプチドとして５ｍｇ～４ｇの範囲が適当である。

【００１２】

【実施例】製造例１

２０リットル容のジャーファーメンター（ケマップ社製）中、ツェイン（和光純薬（株）製）７５０ｇを４℃の脱イオン水１５リットルに懸濁し、２５％アンモニア水（和光純薬（株）製）によってｐＨを７．５に調整した。サーモリシン（大和化成（株）製）７ｇを加え、撹拌しながら６０℃で１８時間反応を行った。これをオートクレーブ（１０５℃、５分）してサーモリシンを失活させた後、遠心分離（６，０００ｒｐｍ、１５分）し、更にポアサイズ０．２ミクロンのメンブレンフィルターで固形物を除去し、凍結乾燥を行いペプチド粉末７４５ｇを得た。

【００１３】試験例１　ＡＣＥ阻害活性の測定

５ｇのラビットラングアセトンパウダー（シグマ社製）を５０ミリリットルの０．１Ｍホウ酸緩衝液（ｐＨ８．３）に溶解し、４０，０００ｘｇで４０分間遠心分離した。その上澄液を上記緩衝液で１０倍に希釈し、アンジオテンシン変換酵素液を得た。検液を試験管に０．０３ミリリットル採り、これに基質として２５０マイクロリットルのヒプリル－Ｌ－ヒスチジル－Ｌ－ロイシン（シグマ社製、最終濃度５ｍＭ、塩化ナトリウム３００ｍＭを含む）を添加し、３７℃で１０分間プレインキュベートした後、上記酵素液を０．１ミリリットル添加し、３７℃で３０分間反応させた。経時後、１Ｎ塩酸０．２５ミリリットルを添加して反応を停止させ、１．５ミリリットルの酢酸エチルを加え、１５秒間激しく撹拌させた。その後、３，５００ｒｐｍで１５分間遠心して、酢酸エチル層１ミリリットルを採取した。その酢酸エチル層より溶媒を減圧除去した。溶媒除去後、蒸留水１ミリリットルを添加し抽出されたヒプリン酸の吸収（２２８ｎｍの吸光度）を測定し、以下の計算式より阻害活性を求めた。５０％の阻害率を示す試料濃度をＩＣ$_{50}$とした。

【００１４】阻害率＝（Ａ－Ｂ）／Ａ×１００％
但し、Ａ：阻害剤を含まない場合の２２８ｎｍの吸光度
Ｂ：阻害剤添加の場合の２２８ｎｍの吸光度
前記製造例１によって調製したトウモロコシ蛋白質の加水分解物のＡＣＥ阻害活性は、ＩＣ５０が２１マイクログラム／ミリリットルであった。

【００１５】試験例２
製造例１で調製したトウモロコシ蛋白質の加水分解物と食塩を含む飼料を自然発症高血圧ラットに自由摂取させ、血圧上昇抑制作用をみた。３週令の自然発症高血圧ラット（日本チャールズリバー社）を温度２３±３℃、湿度５０±１０％の飼育室で１週間予備飼育した後、健常なものを試験に用いた。試験飼料は、製造例１で得たトウモロコシ蛋白質の加水分解物２８％（粗蛋白質量として２４．６％）、コーンスターチ４１．５％、アルファー澱粉１０％、セルロースパウダー８％、植物油６％、ミネラル類３．５％、グラニュー糖５％及びビタミン類１％を混合した「ペプチド配合飼料」を調製し、これとオリエンタル酵母工業（株）製「ＭＦ粉末飼料」を異なる比率で混合した。すなわち「ＭＦ粉末飼料」のみ＊（対照区１）、「ＭＦ粉末飼料」と「ペプチド配合飼料」を２：１に配合したもの（試験区１）、及び同前を１：１に配合したもの（試験区２）である。１試験区は各７匹のラットを用い、試験期間は４週間とした。各供用飼料は食塩量を３．２％に調整、飲水は水道水を用い、いずれも自由に摂取させた。測定は、飼料摂取量、増体重、血圧及び心拍数につき１週間に１度行った。血圧及び心拍数の測定は、非観血式血圧・心拍数測定装置（ユニコム社製　ＴＫ－３５０型）を用い、１匹につき６回測定した値の平均値を求めた。試験結果は、血圧測定結果を図１、飼料摂取量と増体重並びに心拍数の測定結果を表１にそれぞれ示す。

【００１６】図１から明らかなように対照区の自然発症高血圧ラットは食塩の過剰摂取により顕著な血圧上昇を示し、これに対し、本発明のトウモロコシペプチドを配合した飼料を給与した試験区１及び試験区２では有意な血圧の上昇抑制効果が認められた。

【００１７】
【表１】

試験例２における投与４週間後(８週齢時)の測定結果

試験区	平均飼料摂取量 (g/head/day)	増体重 (g/4weeks)	心拍数 (beats/min)
対照区	15.7±0.6	123.3±4.7	457.9±15.5
試験区１	14.3±0.8	125.9±3.0	442.4±9.2
試験区２	14.7±0.6	124.0±2.7	434.7±10.9

【００１８】一方、心拍数は、表１に見られるようにいずれの区も有為な差は認められなかった。また、表１では、飼料摂取量及び増体重も各区間に有意な差はなく、栄養面、安全面でも問題がないことが確認された。

【００１９】試験例３
製造例１で調製したトウモロコシ蛋白質の加水分解物と食塩を含む飼料を、より高齢の自然発症高血圧ラットに自由摂取させ、血圧上昇抑制作用を確認するとともに、血中コレステロール等への影響を調べた。６週令の自然発症高血圧ラット（日本チャールズリバー社）を試験例２と同様に１週間予備飼育した後、健常なものを試験に用いた。飼料はオリエンタル酵母工業（株）製「ＭＦ粉末飼料」のみのもの（対照区２）、及びこれに製造例１で得られたペプチドを１０％に配合したもの（試験区３）とし、ともに終濃度が３．２％になるように食塩を添加した。飲水には水道水をいずれも自由摂取させた。試験例２と同様にして飼料摂取量、増体重、血圧及び心拍数の測定を行った。また、試験終了後、ラットを断頭して血液を摂取後直ちに血液のアンジオテンシンＩＩの定量をラジオイムノアッセイ法により、総コレステロール、中性脂肪、高密度リポ蛋白コレステロールの定量を酵素法により行った。試験結果は、血圧測定結果を図２、飼料摂取量と増体重並びに心拍数の測定結果を表２、血液中のアンジオテンシンＩＩ、血液中の総コレステロール、中性脂肪及び高密度リポ蛋白質の測定値を表３、にそれぞれ示す。

【００２０】図２から明らかなように対照区の自然発症高血圧ラットは食塩の過剰摂取により顕著な血圧上昇を示し、これに対し、本発明のトウモロコシペプチドを配合した飼料を給与した試験群では有意な血圧の上昇抑制効果が認められた。

【００２１】
【表２】

試験例３における投与３週間後（１０週齢時）の測定結果－１

試験区	平均飼料摂取量 (g/head/day)	増体重 (g/3weeks)	心拍数 (beats/min)
対照区２	16.2±0.6	90.6±5.6	452.9±15.5
試験区３	16.0±0.8	88.4±4.8	454.4±9.2

【００２２】　　＊　＊【表３】

試験例３における投与３週間後（１０週齢時）の測定結果－２

試験区	血中ｱﾝｼﾞｵﾃﾝｼﾝＩＩ (ng/ml)	同総コレステロール (mg/ml)	同中性脂肪 (mg/dl)	同HDLｺﾚｽﾃﾛｰﾙ＊ (mg/dl)
対照区２	91.8±18.6	55.3±2.6	140±16	41.3±1.7
試験区３	27.5±5.6	47.0±1.5	120±16	40.0±0.9

注)　*高密度リポ蛋白コレステロール

【００２３】飼料摂取量と増体重並びに心拍数は、試験例２と同様、対照区と試験区間に有意な差は認められなかった。（表２）

血漿アンジオテンシンＩＩ量は、表３に示すように、対照区では著しく増加しているが、本発明のペプチドの配合により有意に低下し、そのレベルは対照区の１／３以下であった。更に血中の総コレステロール及び中性脂肪量は、ともに本発明のトウモロコシペプチドを添加した区で低下効果が認められ、生体にとって有用なＨＤＬコレステロールの低下を伴うことなく、これらを有意に減少させることが分かった。

【００２４】

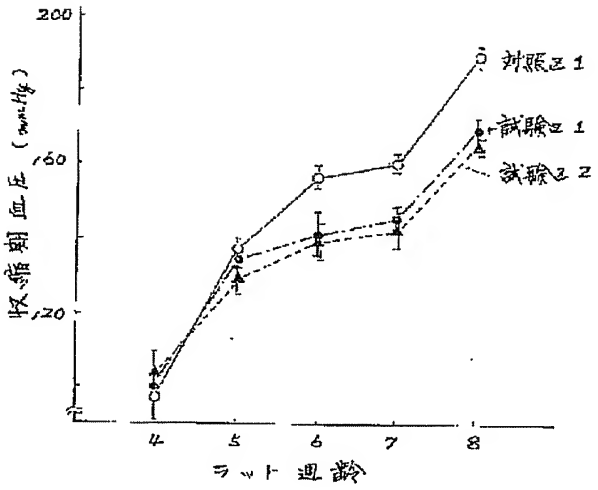
【発明の効果】本発明の高脂血症の治療又は予防用経口剤は血中のコレステロールや中性脂肪の量を有意に低下させる機能を有し、さらにナトリウム塩の有する血圧上昇作用を抑える機能も有する。

【図面の簡単な説明】

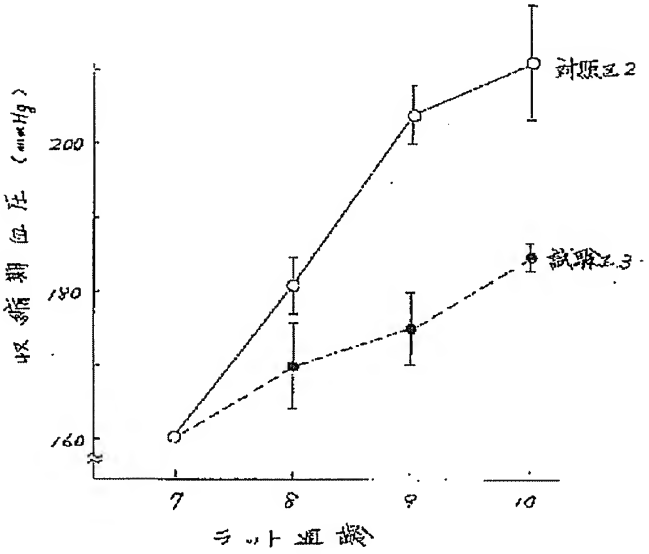
【図１】試験例２における動物実験の血圧測定結果を各群毎の平均値と標準偏差で示すものである。横軸はラットの週令、縦軸はラットの収縮期血圧（ｍｍＨｇ）である。実線は対照区１、１点鎖線は試験区１、点線は試験区２の結果を、それぞれ示している。

【図２】試験例３における動物実験の血圧測定結果を各群毎の平均値と標準偏差で示すものである。横軸はラットの週令、縦軸はラットの収縮期血圧（ｍｍＨｇ）である。実線は対照区２、点線は試験区３の結果を、それぞれ示している。

【図１】



【図２】



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